UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL CONTROL OF THE DIAMONDBACK MOTH (PLUTELLA XYLOSTELLA LINNAEUS) USING PLUTELLA XYLOSTELLA GRANULOVIRUS

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By

AHMAD DEZIANIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2011

DEDICATION

I would like to dedicate my thesis to my late father, my dear mother and beloved spouse who always inspired and supported me to achieve this goal

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

BIOLOGICAL CONTROL OF THE DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA* LINNAEUS) USING *PLUTELLA XYLOSTELLA* GRANULOVIRUS

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February 2011

Chairman: Professor Ahmad Said Sajap, PhD

Faculty: Forestry

The diamondback moth (DBM) *Plutella xylostella*, is known to be the most serious pest of crucifers in the world. Regular and repeated use of chemical insecticides for controlling the pest over the years has resulted in DBM developing resistance to all classes of chemical insecticides. These shortcomings have prompted researchers to evaluate the potential of biological control of DBM as an alternative to conventional insecticides. One of the biological control agents is *Plutella xylostella* granulovirus. *Plutella xylostella* granulovirus (*Px*GV) was effective in suppressing the Malaysian population of DBM larvae. The virus was originally isolated from Taiwan and was confirmed by Restriction Endonuclease (REN) analysis. The pattern and DNA profile of the GV was compared with that of *Px*GV isolates reported earlier. Scanning Electron Micrograph revealed that the capsules of *Px*GV were ovocylindrical with a mean size of 272.84 ± 12 (length) × 148.27 ± 19 (width) nm. The virions were 168.44 ± 16 (length) × 29.57 ± 12 (width) nm and rod-shaped. Results from bioassay showed that larval mortality was significantly influenced by larval instar and viral concentrations. Mortalities caused by the highest virus

concentration (3.11×10^9 Granules/ml) were 98.30 %, 96.60 %, 73.30 %, and 14.30 %, in the first to fourth instar, respectively. Larval mortality increased with increasing viral concentrations and decreased with the older instars. The median lethal concentration (LC_{50}) in the second instars was estimated 1.39×10^6 Granules/ml.

*Px*GV was susceptible to ultra violet radiation (UV) and lost almost all of its infectivity after 7 hours of exposure to UV-B radiation under laboratory conditions. Virulency of UV treated *Px*GV were reduced when compared to non-treated *Px*GV as much as 19.64%, 41.53%, 63.17%, 70% and 89 % after 5, 15, 30, 60 and 120 minutes exposure to UV radiation, respectively. Adjuvants consisting of Tinopal, molasses, lignin and skimmed milk added separately to *Px*GV suspension significantly improved the residual activity of *Px*GV after exposure to UV radiation. *Px*GV + Tinopal, *Px*GV + molasses, *Px*GV + lignin and *Px*GV + skimmed milk increased residual activity 67.78%, 65.31%, 59.55% and 31.35% after being exposed to UV radiation, respectively. The molasses and Tinopal when incorporated with different virus concentrations before exposure to UV light significantly increased the residual activity. Molasses+virus showed greatest effects on the larval mortality at all virus concentrations compared to those of Tinopal+virus and lignin+virus before exposure to UV light. The LC_{50} calculated for virus + molasses (5.2×10^4 Granules/ml) before exposure to UV light was 9.2 and 1.75 times lower than lignin+virus and Tinopal+virus respectively.

Four spray-dried formulations were produced using a spray drying machine (Mini Spray Dryer Buchi-B 290) at concentration of 1.4×10^{10} Granules/g and coded as

CLM (*Px*GV + modified food starch + lignin + molasses), KLM (*Px*GV + kaolin + lignin + molasses), CAP (*Px*GV + modified food starch) and KAL (*Px*GV + kaolin). These formulations, except KAL, caused higher larval mortality than the unformulated virus before exposure to UV light. The CLM formulation caused the highest mortality (98.60%) after exposure to UV light and significantly improved the residual activity compared to the unformulated virus. KLM, CAP, KAL formulations and control treatment provided 87.13%, 64.87%, 45.97% and 27.06% larval mortality, respectively. The LC_{50} values estimated for CLM formulations (1.7×10^5 Granules/ml) was 1.76 fold lower than KLM formulation. The results indicate that spray drying procedure did not cause adverse effects on the virus particles. The encapsulated formulation not only provided effective protection to occlusion bodies against UV radiation but it also improved biological activity of the virus as well. The spray-dried ingredients and size of microgranules considerably improved CLM formulation suspensibility. The results of semi-field tests showed that CLM formulation considerably improved residual activity of the *Px*GV under natural conditions. After 0.5, 5, 24, 48 and 72 hours of exposure to the natural condition, virus of the CLM formulation still retained 13.7%, 30.67%, 42.93%, 52.53% and 21.76% compared to unformulated virus, respectively. CLM formulation was significantly more effective on larval mortality than KLM formulation after exposure to natural conditions. Although spray dried formulation of *Px*GV was effective for controling of the pest both in the laboratory and semi-field tests, further field experiment and economical feasibility study are recommended.

Abstrak of tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGAWALAN BIOLOGI BAGI DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*.LINNAEUS) DENGAN MENGGUNAKAN *PLUTELLA XYLOSTELLA* GRANULOVIRUS

Oleh

AHMAD DEZIANIAN

Febuari 2011

Pengerusi: Profesor Ahmad Said Sajap, PhD

Fakulti: Perhutanan

Diamondback moth (DBM) *Plutella xylostella* dikenali sebagai perosak yang sangat serius bagi sayur-sayuran di dunia. Penggunaan racun kimia untuk pengawalan serangga perosak yang bertahun-tahun lamanya, telah memberikan keputusan dalam peningkatan sistem pertahanan DBM kepada semua pengkelasan bagi racun kimia. Situasi ini telah mendorong para penyelidik mengambil langkah segera untuk menilai potensi pengawalan biologi bagi DBM sebagai langkah alternatif racun kimia. Salah satunya adalah *P. xylostella* granulovirus (*Px*GV) sebagai agen pengawalan biologi yang berkesan untuk mengawal populasi bagi larva DBM di Malaysia. Virus ini berasal dari Taiwan dan telah disahkan oleh analisis Restriction Endonuclease (REN). Size genome GV telah dipatenkan berbanding dengan isolate *Px*GV yang telah dilaporkan. Melalui pemerhatian Scanning Electron Micrograph, dari segi ciri morphologi, *Px*GV telah mempamerkan kapsulnya berbentuk ovocylindrical dengan bersaiz 272.84 ± 12 × 148.27 ± 19 nm. Manakala virion-virionnya adalah bersaiz 168.44 ± 16 × 29.57 ± 12 nm dan berbentuk rod. Keputusan

bioassay telah mempamerkan kematian larva adalah significant berdasarkan peringkat larva dan kepekatan viral. Kematian disebabkan kepekatan virus yang tinggi (3.11×10^9 Granules/ml) di mana 98.3%, 96.6%, 73.3%, dan 14.3 % daripada instar pertama hingga keempat masing-masing. Kematian larva meningkat dengan peningkatan kepekatan dan pengurangan bagi instar yang semakin matang. Pertengahan lethal kepekatan (LC_{50}) dalam instar kedua adalah dianggarkan 1.39×10^6 Granules/ml.

*Px*GV adalah sensitif dibawah ultra ungu dan kebanyakkannya hilang sifat kepathogenan selepas terdedah selama tujuh jam. Kehilangan sifat kepathogenan virus masing-masing telah mencatat 19.64%, 41.5%3, 63.17%, 70% and 89 % selepas terdedah selama 5, 15, 30, 60 hingga 120 minit di bawah radiasi UV. Bahan-bahan yang meliputi Tinopal, molasses, lignin dan susu skim ditambah secara berasingan pada *P. xylostella* granulovirus (*Px*GV) adalah signifikan dengan peningkatan aktiviti bagi residual *Px*GV selepas terdedah di bawah radiasi UV. PxGV + Tinopal, PxGV + molasses, PxGV + lignin dan susu skim + *Px*GV meningkat 67.78%, 65.31%, 59.55% dan 31.35 % aktiviti residualnya selepas terdedah di bawah radiasi UV masing-masing. Gabungan Molasses dan Tinopal pada kepekatan virus yang berbeza sebelum terdedah di bawah sinaran UV adalah significant bagi peningkatan aktiviti residual. Molasses + virus menunjukkan kesan yang sangat memberansangkan bagi kematian larva pada kesemua kepekatan berbanding dengan Tinopal + virus dan lignin + virus sebelum terdedah di bawah sinaran cahaya UV. LC_{50} menunjukkan virus + molasses (5.2×10^4 Granules/ml) adalah 9.2 dan 1.75 kali kurang daripada lignin + virus dan Tinopal + virus masing-masing sebelum terdedah di bawah sinaran UV.

Empat formula semburan pengeringan telah dihasilkan menggunakan mesin semburan pengeringan (Mini Spray Dryer Buchi-B 290) pada kepekatan 1.4×10^{10} OB/g dan dikodkan sebagai CLM (*Px*GV + modified food starch + lignin + molasses), KLM (*Px*GV + kaolin + lignin + molasses), CAP (*Px*GV + modified food starch) dan KAL (*Px*GV + kaolin). Formula-formula tersebut kecuali KAL menunjukkan peningkatan dalam kematian larva berbanding virus tanpa formula sebelum terdedah di bawah sinaran cahaya UV. Formula CLM menunjukkan kematian yang tinggi (98.60%) selepas terdedah di bawah sinaran UV dan significant bagi peningkatan aktiviti residual berbanding dengan virus tanpa formula. Manakala formula KLM, CAP, KAL dan rawatan kawalan masing-masing menghasilkan 87.13%, 64.87%, 45.97% dan 27.06 % bagi kematian larva. Nilai LC_{50} dianggarkan untuk formula CLM adalah (1.7×10^5 Granules/ml) 1.76 bahagian adalah kurang daripada formula KLM. Keputusan-keputusan tersebut mendapati prosedur penyemburan kering tidak memberi sebarang kesan terhadap partikel virus. Lagipun formula encapsula bukan hanya menghasilkan perlindungan kepada jasad virus di bawah radiasi UV yang banyak tetapi juga meningkatkan aktiviti biologi yang sangat baik bagi virus. Kandungan semburan pengeringan dan saiz bagi microgranul didapati memberi peningkatkan suspensibiliti bagi formula CLM. Keputusan-keputusan bagi ujian separuh lapangan menunjukkan peningkatan aktiviti residual formula CLM bagi *Px*GV di bawah keadaan semulajadi. Selepas terdedah selama 0.5, 5, 24, 48 dan 72 jam kepada keadaan semulajadi, virus bagi formula CLM masih mengekalkan 13.7%, 30.67%, 42.93%, 52.53% dan 21.76 % aktiviti residualnya berbanding dengan tanpa formula masing-masing. Formula CLM adalah significant dan sangat berkesan terhadap kematian larva berbanding formula KLM selepas terdedah pada keadaan semulajadi. Walaupun formula penyemburan kering *Px*GV

menunjukkan keberkesanan yang menarik untuk mengawal serangga perosak baikpun di dalam makmal dan separa lapangan, eksperimen di lapangan dan kemampuan ekonomi dicadangkan.

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I certify that a Thesis Examination Committee has met on ……to conduct the final examination of Ahmad Dezianian on his thesis entitled "Biological Control of Diamondback Moth (*Plutella xylostella* Linnaeus) Using *Plutella xylostella* granulovirus" with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

AHMAD DEZIANIAN

Date: 22 February 2011

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